## Phylogeny

A-Raf is one of three vertebrate Raf paralogs (A-Raf, B-Raf, C-Raf) that arose by duplication of a single Raf ortholog present in invertebrates such as LIN-45 (Caenorhabditis elegans) and D-Raf (Drosophila melanogaster) (marais1997differentialregulationof pages 1-1).  
Documented orthologs include mouse A-Raf, rat A-Raf, Xenopus araf, zebrafish araf, LIN-45, and D-Raf, confirming deep evolutionary conservation (unknownauthors2010araf pages 1-3).  
Within the human kinome, A-Raf is classified in the tyrosine-kinase-like (TKL) group, MAP3K family, Raf subfamily (an2015arafanew pages 12-12).  
B-Raf is phylogenetically oldest and has the highest basal catalytic activity, whereas A-Raf is the most divergent isoform and exhibits the lowest intrinsic MEK kinase output (an2015arafanew pages 2-3).

## Reaction Catalyzed

ATP + protein-L-Ser/Thr ⇌ ADP + protein-L-Ser/Thr-phosphate (lavoie2015regulationofraf pages 1-2).

## Cofactor Requirements

Catalytic turnover requires divalent Mg²⁺ (unknownauthors2010araf pages 3-5).

## Substrate Specificity

Physiological substrates are MEK1 and MEK2; A-Raf displays approximately 20 % of C-Raf maximal activity and shows a cell-type-dependent preference for MEK1 over MEK2 (unknownauthors2010araf pages 1-3).  
A unique consensus phosphopeptide motif has not been defined beyond recognition of the canonical MEK activation-loop serine residues, and no additional broad peptide specificity has been reported (unknownauthors2010araf pages 11-12).

## Structure

Domain organization: CR1 contains the Ras-binding domain and cysteine-rich domain responsible for Ras engagement and membrane recruitment (an2015arafanew pages 2-3).  
CR2 is a serine/threonine-rich regulatory segment bearing the internal 14-3-3 site at Ser214 together with multiple inhibitory phosphosites (an2015arafanew pages 2-3).  
CR3 is the C-terminal kinase domain that houses the activation-segment residues Thr452 and Thr455 and the C-terminal 14-3-3 site at Ser582 (an2015arafanew pages 3-5).  
Three-dimensional arrangement follows the side-to-side dimer architecture observed in B-Raf:14-3-3 and B-Raf:MEK complexes, featuring inward movement of the αC-helix and alignment of regulatory and catalytic spines upon activation (kondo2021newinsightsinto pages 1-3).  
Key catalytic motifs include the HRD catalytic loop, DFG motif, activation segment, and AS-H1 helix that stabilises the active state (lavoie2015regulationofraf pages 8-9).  
A-Raf possesses a unique Tyr296 in the N-region that restrains basal activity; mutation or phosphorylation of neighboring Tyr301/Tyr302 elevates catalytic output (an2015arafanew pages 3-5).  
Ser257, Ser262 and Ser264 in the internal hinge modulate electrostatic interactions with membranes and influence subcellular localisation (unknownauthors2010araf pages 3-5).

## Regulation

Phosphorylation at Ser214 creates a high-affinity 14-3-3 docking site that suppresses kinase activity (an2015arafanew pages 2-3).  
Phosphorylation of Ser582 constitutes a secondary 14-3-3 site that is dispensable for activation (an2015arafanew pages 2-3).  
Ser432 phosphorylation is essential for productive MEK binding and catalysis (unknownauthors2010araf pages 1-3).  
Activation-segment Thr452 and Thr455 are required for maximal Ras12V/Lck-driven activation (an2015arafanew pages 3-5).  
Phosphorylation of Ser257, Ser262 and Ser264 promotes dissociation from the plasma membrane (unknownauthors2010araf pages 3-5).  
Src family kinases phosphorylate Tyr301 and Tyr302 to enhance activity, whereas Tyr296 acts as a negative regulatory residue (lavoie2015regulationofraf pages 8-9).  
CK2β binds A-Raf and markedly augments its catalytic activity (unknownauthors2010araf pages 3-5).  
mTORC2 directly phosphorylates A-Raf, modulating Smad2 signalling (an2015arafanew pages 8-9).  
14-3-3 dimers stabilise either inactive monomeric or active dimeric states depending on phosphosite occupancy (kondo2021newinsightsinto pages 1-3).  
Autoinhibition is relieved by Ras-GTP binding to CR1, lipid-dependent membrane anchoring and kinase-domain dimerisation (lavoie2015regulationofraf pages 8-9).

## Function

A-Raf is abundantly expressed in epididymis, ovaries, liver, uterus and kidney, with low expression in neural tissues (an2015arafanew pages 2-3).  
The protein localises to cytoplasm, translocates to the plasma membrane upon Ras activation, and is also found at mitochondria and tubular endosomes (unknownauthors2010araf pages 5-7).  
Upstream activators include GTP-loaded Ras, Gα12, platelet-derived growth factor receptor signalling via p85 PI3K SH2 interaction, and membrane phosphoinositides (an2015arafanew pages 8-9).  
Downstream effectors comprise MEK1/2-ERK, inhibition of MST2 apoptotic kinase, tetramerisation of pyruvate kinase M2, and phosphorylation of PKCδ via an mTORC2 route (unknownauthors2010araf pages 5-7).  
Biological roles encompass promotion of cell proliferation and migration, suppression of apoptosis, regulation of aerobic glycolysis, and participation in endocytic membrane traffic (mooz2014dimerizationofthe pages 6-7).

## Other Comments

Somatic activating ARAF mutations are documented in lung adenocarcinoma and operate through RAS-independent dimerisation mechanisms (su2022arafproteinkinase pages 3-5).  
A-Raf over-expression is reported in astrocytic, head-and-neck, colon and pancreatic carcinomas (unknownauthors2010araf pages 5-7).  
A-Raf knockout mice display post-natal lethality with neurological and intestinal defects, indicating non-redundant developmental functions (unknownauthors2010araf pages 5-7).  
Alternative splice variants DA-Raf1 and DA-Raf2 retain CR1 but lack the kinase domain, acting as dominant-negative inhibitors of the Ras-ERK pathway and influencing myogenic differentiation (an2015arafanew pages 3-5).

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